Biochimica et Biophysica Acta 1812 (2011) 488–494

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Biophysical properties of mutant KCNQ1 S277L channels linked to hereditary long QT syndrome with phenotypic variability

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ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 6 January 2011
Accepted 10 January 2011
Available online 15 January 2011

Keywords:
Cardiac electrophysiology
I_{Ks} current
KCNQ1 (KVLQT1)
Long QT syndrome
Mutation
Sudden cardiac death

ABSTRACT

Hereditary long QT syndrome (LQTS) is associated with ventricular torsade de pointes tachyarrhythmias and sudden cardiac death. Mutations in a cardiac voltage-gated potassium channel, KCNQ1, induce the most frequent variant of LQTS. We identified a KCNQ1 missense mutation, KCNQ1 S277L, in a patient presenting with recurrent syncope triggered by emotional stress (QTc = 528 ms). This mutation is located in the conserved S5 transmembrane region of the KCNQ1 channel. Using *in vitro* electrophysiological testing in the *Xenopus* oocyte expression system, the S277L mutation was found to be non-functional and to suppress wild type currents in dominant-negative fashion in the presence and in the absence of the regulatory ß-subunit, KCNE1. In addition, expression of S277L and wild type KCNQ1 with KCNE1 resulted in a shift of the voltage-dependence of activation by −8.7 mV compared to wild type I_{Ks}, indicating co-assembly of mutant and wild type subunits. The electrophysiological phenotype corresponds well with the severe clinical phenotype of the index patient. However, investigation of family members revealed three patients that exhibit asymptomatic QT interval prolongation (QTc = 493–518 ms). In conclusion, this study emphasizes the value of biophysical testing to provide mechanistic evidence for pathogenicity of ion channel mutations identified in LQTS patients. The inconsistent association of the KCNQ1 S277L mutation with the clinical presentation suggests that additional genetic, epigenetic, or environmental factors play a role in defining the individual clinical LQTS phenotype.

1. Introduction

Hereditary long QT syndromes (LQTS) are cardiac repolarization abnormalities characterized by a prolonged QT interval on the surface ECG. LQTS are associated with syncope and a high risk of sudden cardiac death due to ventricular tachyarrhythmias [1]. More than 700 mutations in twelve different genes have been described to date [2]. LQTS1, the most frequent form of LQTS, is associated with KCNQ1 (KvLQT1) gene mutations [3]. Co-expression of the pore-forming α-subunit KCNQ1 and its regulatory β-subunit KCNE1 (minK) elicits slowly activating potassium currents, resembling the slow component of the cardiac delayed rectifier potassium current I_{Ks} [4,5]. I_{Ks} contributes to cardiac repolarization and is a target for class III antiarrhythmic drugs such as dronedarone [6]. Loss-of-function mutations in KCNQ1 lead to prolonged cardiac repolarization and cause congenital long QT syndrome 1 [7–12]. In contrast, KCNQ1 gain-of-function mutations may induce short QT syndrome [13] or hereditary atrial fibrillation [14].

Previous work on genotype–phenotype correlations has indicated that the individual LQTS genotype influences the clinical course [15,16]. Approximately one third of KCNQ1 mutations identified in LQTS patients are located in the pore region or in adjacent transmembrane regions, S5 and S6 [17]. Carriers of these mutations exhibit a higher risk for cardiac events and tend to be affected at younger ages compared to patients with mutations in the C-terminal region [18,19]. Furthermore, Moss et al. [20] revealed that location of KCNQ1 mutations in the pore and transmembrane regions and dominant-negative current suppression *in vitro* may serve as predictors of severe clinical courses independent of traditional clinical risk factors, suggesting potential significance for therapy planning in LQTS patients.

In the present study, we analyse biophysical properties of mutant KCNQ1 S277L potassium channels identified in a German LQTS family. This mutation is located in the S5 transmembrane region of the KCNQ1 channel and causes loss of channel function. However, closely related family members display phenotypic variability ranging from asymptomatic QTc prolongation to recurrent ventricular tachycardia despite harbouring the same mutation associated with a severe cellular phenotype. We conclude that modifying factors contribute to the individual clinical phenotype to a larger extent than previously appreciated.

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doi:10.1016/j.bbadis.2011.01.008

2. Materials and methods

2.1. Pedigree and mutation analysis

Clinical evaluation and blood samples are based on an LQTS family of German origin (Fig. 1A). The index patient (II.4, female) was brought to our attention after recurrent syncope and aborted sudden death had occurred during emotional stress. The investigation conforms to the principles outlined in the Declaration of Helsinki. Informed consent was obtained for genetic analyses. DNA sequence analyses were performed by the Center for Human Genetics and Laboratory Medicine (Martinsried, Germany).

2.2. Molecular biology

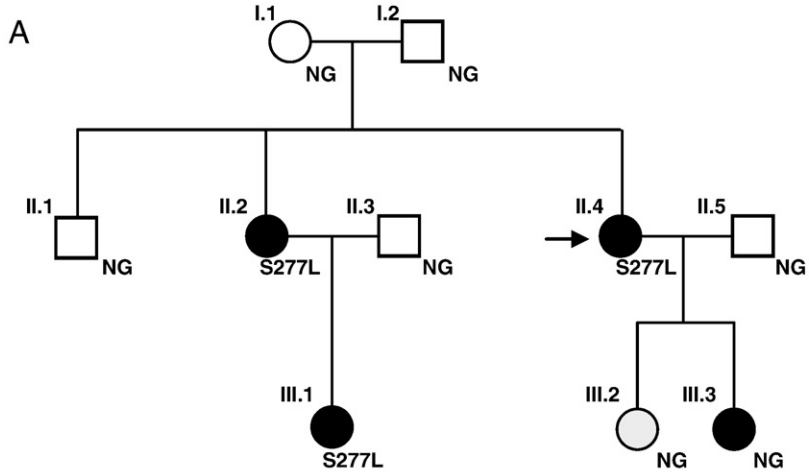
Complementary DNA encoding human KCNQ1 and human KCNE1 was kindly provided by Dr. Steve Goldstein (Chicago, USA). To introduce the S277L mutation into KCNQ1, site-directed PCR-mutagenesis was performed using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, USA) as described [21], and resulting cDNA was analysed by DNA sequence analysis.

2.3. Heterologous gene expression in Xenopus laevis oocytes

Procedures for *in vitro* transcription and oocyte injection have been published previously [9]. Briefly, WT KCNQ1, KCNQ1 S277L, and KCNE1 cRNAs were prepared with the mMESSAGE mMACHINE kit (Ambion, Austin, USA) using T7 RNA polymerase. Stage V–VI defolliculated *Xenopus* oocytes were injected with 46 nl of cRNA per oocyte, and electrophysiological measurements were performed 2 days after injection. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996). The European Community guidelines for the use of experimental animals have been adhered to.

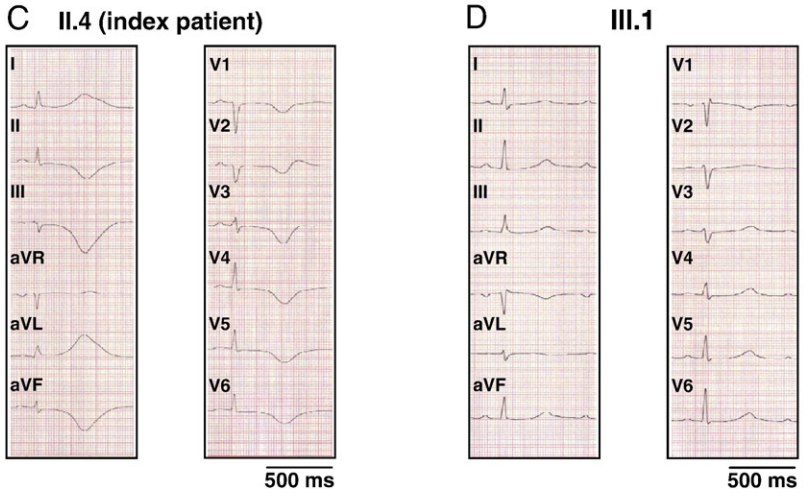
2.4. Electrophysiology and data analysis

Two-microelectrode voltage-clamp recordings from *Xenopus laevis* oocytes were carried out as published previously [9]. Voltage clamp measurements of *Xenopus* oocytes were performed in a solution containing (in mM): 5 KCl, 100 NaCl, 1.5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH 7.4, adjusted with NaOH). Current and voltage electrodes were filled with 3 M KCl solution. All experiments were carried out at room



B

Patient ID	Age (years)	Sex	QTc (ms)	LQTS score	Trigger of syncope
II.2	50	F	511	4	n/a
II.4	49	F	528	5.5	emotion
III.1	19	F	493	4	n/a
III.3	11	F	518	4	n/a



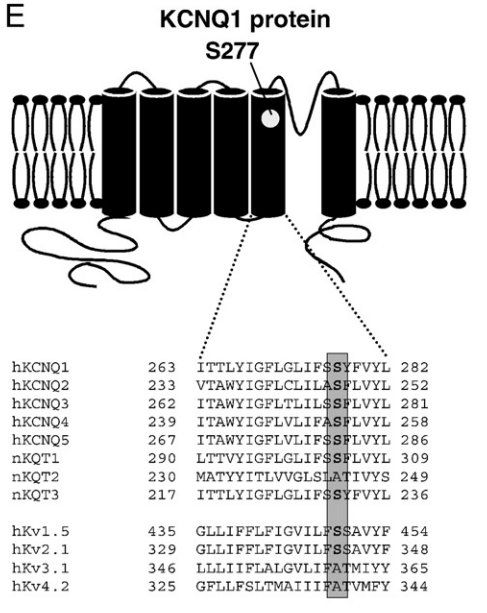


Fig. 1. Patient characteristics and identification of the KCNQ1 S277L mutation. (A) pedigree of the LQTS family analysed in this study (arrow indicates the index patient). Closed symbols denote definite long QT syndrome (i.e., Schwartz Score ≥ 4 [1,23]), open symbols indicate unaffected family members. Circles refer to women, squares indicate men. S277L, heterozygous S277L genotype; NG, not genotyped. (B) clinical evaluation of LQTS family members. (C, D) resting ECGs of the index patient (II.4; C) and patient III.1 (D) reveal significant QTc prolongation. (E) membrane folding model of the KCNQ1 protein, indicating the location of the mutated amino acid residue, S227. Residue S277 is highly conserved, as demonstrated by amino acid sequence alignments of S5 transmembrane segments of human KCNQ family potassium channels KCNQ1-5, *Caenorhabditis elegans* KCNQ homologs KQT1-3, and select human voltage gated potassium (Kv) channels. S277 and its homologs are boxed. The respective GenBank accession numbers are AF000571, NM_172107, NM_004519, NM_004700, and NM_019842 for KCNQ1-5, NM_171709, NM_076991, and NM_064474 for KQT1-3, and NM_002234 (hKv1.5), NM_004974 (hKv2.1), NM_004976 (hKv3.1), NM_012281 (hKv4.2).

temperature (20–22 °C). To assess half-maximal activation voltages, activation curves were fit with a Boltzmann distribution: $G(V) = G_{max}/(1 - \exp[(V_{1/2} - V)/k])$, where V is the test pulse potential, $V_{1/2}$ is the half-maximal activation potential, and k is the slope of the activation curve.

2.5. Statistics

Data are expressed as mean ± standard error of the mean (SEM). Unpaired Student's t tests (two-tailed tests) were used to compare the statistical significance of the results: $p<0.05$ was considered statistically significant. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05-level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction.

3. Results

3.1. Clinical analysis and genetics

The index patient (II.4; Fig. 1A) initially presented with a history of recurrent syncope, ventricular tachycardia, and aborted sudden death triggered by emotional arousal. The resting ECG with a QTc interval of 528 ms (Fig. 1B and C) led to the diagnosis of long QT syndrome. She received an implantable cardioverter defibrillator (ICD) and was treated with ß-blockade, under which the patient continued to display recurrent episodes of ventricular tachycardia. Prolonged QT intervals were detected in three additional female family members (Fig. 1A, B and D) as well. In contrast to the index patient, these probands did not report any clinical symptoms of long QT syndrome (Fig. 1B). In order to identify the underlying mutation, genomic DNA from the index patient and from two additional family members was sampled. DNA sequence analyses revealed C to T transversions at nucleotide position 830 of all patients available for genetic assessment. As a result, a conserved serine residue was replaced by leucine at position 277 located in the S5 transmembrane segment of the KCNQ1 ion channel polypeptide (Fig. 1E).

3.2. Electrophysiological analysis of mutant KCNQ1 S277L channels

Homomultimeric channels formed by wild type (WT) or mutant KCNQ1 S277L subunits were functionally characterized in *Xenopus* oocytes injected with the respective cRNA (23 ng each). Currents were activated during depolarizing steps to potentials ranging from −60 mV to +120 mV (2 s), and tail currents were recorded at −40 mV (2 s). The holding potential was −80 mV, and pulses were applied at a frequency of 0.2 Hz. This voltage protocol was used in all electrophysiological measurements in this work. Tail currents represent KCNQ1 channel activity, while outward currents during the first step of the voltage protocol may in part reflect low endogenous *Xenopus* currents (compare Fig. 2A and D). Thus, tail current amplitudes were analysed. Mean WT KCNQ1 current amplitudes yielded 0.61 ± 0.03 μA (n = 21; Fig. 2A and E), while virtually no tail current was detected in oocytes injected with mutant KCNQ1 S277L cRNA (0.01 ± 0.01 μA; n = 21; Fig. 2B and E).

KCNQ1-associated long QT syndrome is inherited in an autosomal-dominant manner, and affected individuals carry one WT and one mutant KCNQ1 allele. Dominant inheritance might be explained either by haploinsufficiency or by dominant-negative effects of mutant subunits on their WT counterparts. To examine whether KCNQ1 S277L subunits cause dominant-negative current suppression, WT KCNQ1 cRNA (11.5 ng, i.e. 50% of the amount injected in Fig. 2A) and mutant KCNQ1 S277L cRNA (11.5 ng, i.e. 50% of the amount injected in Fig. 2B) were co-injected into oocytes, and the resulting currents (Fig. 2C) were compared with WT KCNQ1 currents (Fig. 2A). Mean KCNQ1 current amplitudes were reduced by 88.5% to 0.07 ± 0.01 μA (n = 21; Fig. 2C and E) compared to WT KCNQ1 currents, indicating that dominant-negative suppression of WT KCNQ1 causes current reduction in the absence of KCNE1 β-subunits.

3.3. KCNQ1 S277L causes dominant-negative I_{Ks} suppression

In human cardiomyocytes, the pore forming α-subunit KCNQ1 and the ß-subunit KCNE1 form channels with characteristics largely similar to I_{Ks} [4,5]. To determine whether the mutants exert a dominant-negative effect in the presence of KCNE1 subunits as well, we co-expressed WT and mutant KCNQ1 S277L subunits in equimolar amounts. The resulting outward potassium current after injection of WT KCNQ1 (11.5 ng) and KCNE1 (11.5 ng) cRNA displayed properties nearly identical to those of native cardiac I_{Ks}, with mean tail current amplitudes of 1.10 ± 0.12 μA (n = 36; Fig. 3A and F). In contrast, I_{Ks} amplitudes of channels composed of KCNQ1 S277L (11.5 ng; 0.01 ± 0.01 μA; n = 36; Fig. 3B and F) with KCNE1 were not significantly different from non-injected oocytes (0.01 ± 0.01 μA; n = 36; Fig. 3E and F). However, co-expression of KCNE1 (11.5 ng) with a combination of KCNQ1 S277L and WT cRNA (5.75 ng each) yielded currents of 0.20 ± 0.03 μA (n = 36; Fig. 3C and F). Thus, S277L reduced I_{Ks} by 81.9% compared to WT currents (Fig. 3F). These data indicate that co-assembly of WT KCNQ1 and mutant S277L subunits results in a dominant-negative effect on I_{Ks}, reflecting a severe cellular phenotype. The activation I–V relationships for KCNQ1/KCNE1 currents (Fig. 4A and B) reveal an 8.7 mV leftward shift in the half-maximal activation voltage in the presence of KCNQ1 S277L (WT KCNQ1 + KCNE1: $V_{1/2} = 82.2 \pm 1.2$ mV; WT KCNQ1 + KCNQ1 S277L + KNCE1: $V_{1/2} = 73.5 \pm 1.3$ mV; n = 15). In all other experiments in this study a reasonable analysis of activation curves was prevented by low I_{Ks} tail current amplitudes.

4. Discussion

A KCNQ1 point mutation was identified in German LQTS1 family, resulting in replacement of a highly conserved serine residue located in the S5 transmembrane region of the channel by leucine (KCNQ1 S277L; Fig. 1E). Electrophysiological characterisation revealed that KCNQ1 S277L subunits did not produce functional potassium channels and suppressed WT KCNQ1 in dominant-negative manner in the presence and in the absence of KCNE1. The half-maximal activation curve of I_{Ks} produced by co-expression of WT KCNQ1 and KCNQ1 S277L with KCNE1 was shifted towards more negative potentials, indicating direct interaction of wild type and mutant subunits. This activation shift in isolation is expected to result in increased current amplitudes at a given membrane potential. However, current increase was not observed owing to strong current suppression by mutant KCNQ1.

The severe electrophysiological phenotype may be readily explained by the location of the affected amino acid. KCNQ1 mutations in the S5-pore-S6 region of the channel generally appear to be associated with more severe clinical presentation [22]. The serine residue at position 277, which is located next to the extracellular surface of the S5 segment in close proximity to the pore helix, is highly conserved in the family of KCNQ channels and among other potassium channels (Fig. 1E). KCNQ1 mutations replacing serine 277 are predicted to cause changes in the pore architecture that significantly affect ion conductance and result in channel dysfunction. Our experimental data revealed that replacement of S277 by leucine prevented KCNQ1 channel function, confirming the hypothesis that this residue is essential for structural integrity and function of the KCNQ1 pore complex. Furthermore, Gouas et al. reported KCNQ1 trafficking inhibition caused by mutation of residue S276 [23]. This suggests that a similar mechanism may contribute to the cellular phenotype associated with the KCNQ1 S277L mutation.

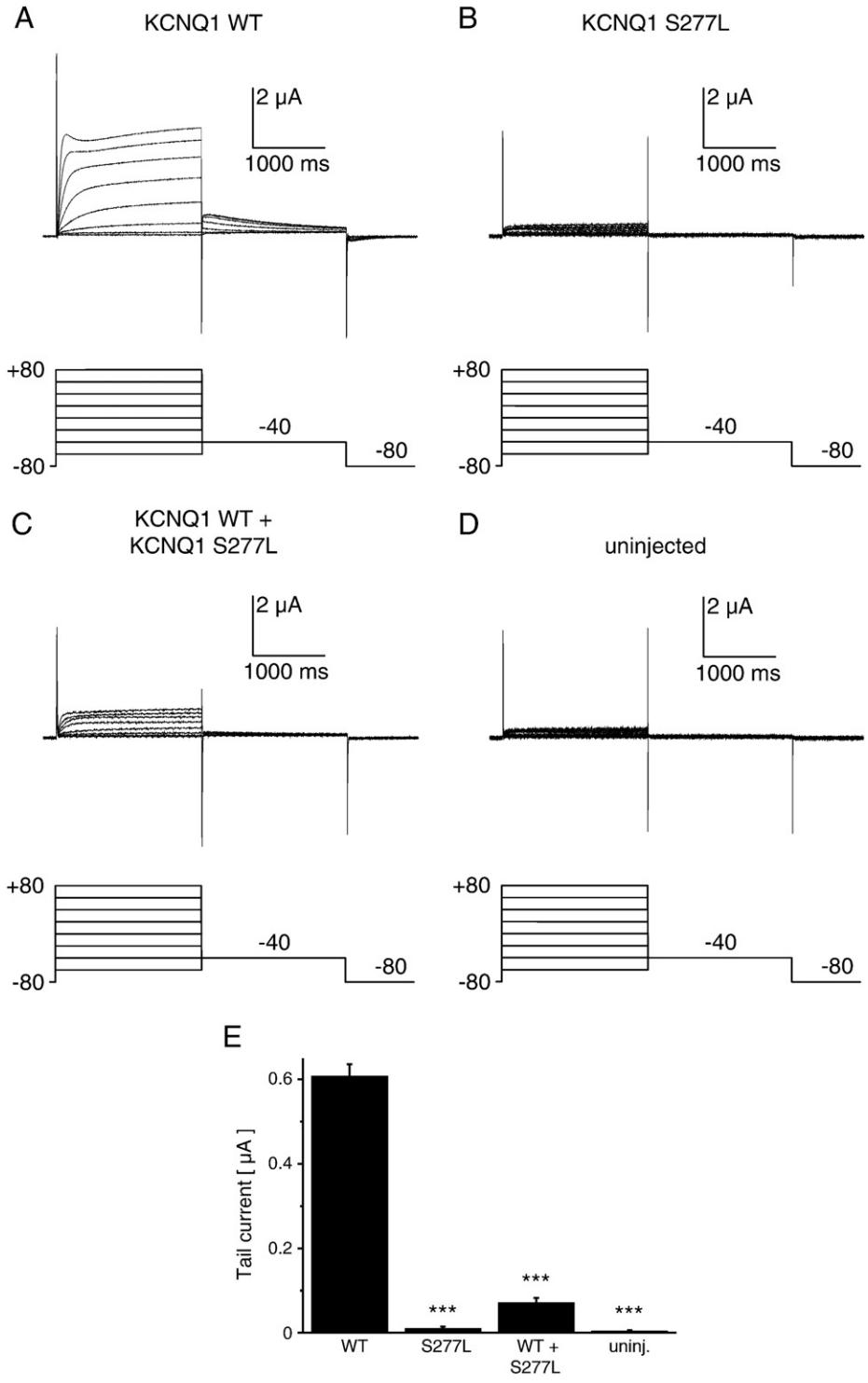


Fig. 2. Currents induced by expression of KCNQ1 α-subunits in *Xenopus* oocytes. Representative current traces recorded from cells expressing WT KCNQ1 (A) or KCNQ1 S277L (B) channels are displayed. (C) currents induced by co-expression of equal amounts of WT KCNQ1 and KCNQ1 S277L subunits. (D) currents recorded from a non-injected control cell. (E) mean peak tail current amplitudes (n = 21 cells per column; ***$p<0.001$ compared to WT KCNQ1). For the purpose of clear presentation, currents recorded at 100 mV and 120 mV are not displayed in panels A to D.

Patients with KCNQ1 mutations located in transmembrane regions of the channel exhibit an increased risk of cardiac arrhythmias compared to patients with mutations in the C-terminus [18–20]. Furthermore, dominant-negative *in vitro* effects of KCNQ1 mutations have recently been identified as an independent risk factor for more severe clinical courses of LQTS [20]. Here, the index patient carrying the KCNQ1 S277L mutation was sensitive to stress-induced ventricular arrhythmia. She exhibited a relatively long QTc interval and a severe clinical phenotype (recurrent syncope and tachycardia, aborted sudden death). This corresponds well to the cellular phenotype, i.e. inactive ion channels and dominant-negative I_{Ks} suppression. In contrast, we identified three asymptomatic family members with LQTS (Schwartz-Score ≥ 4 [1,24]), indicating that genotype–phenotype correlations should be handled with care since I_{Ks} suppression evaluated in heterologous expression systems may not always correlate well with severity of the clinical phenotype [25]. This notion

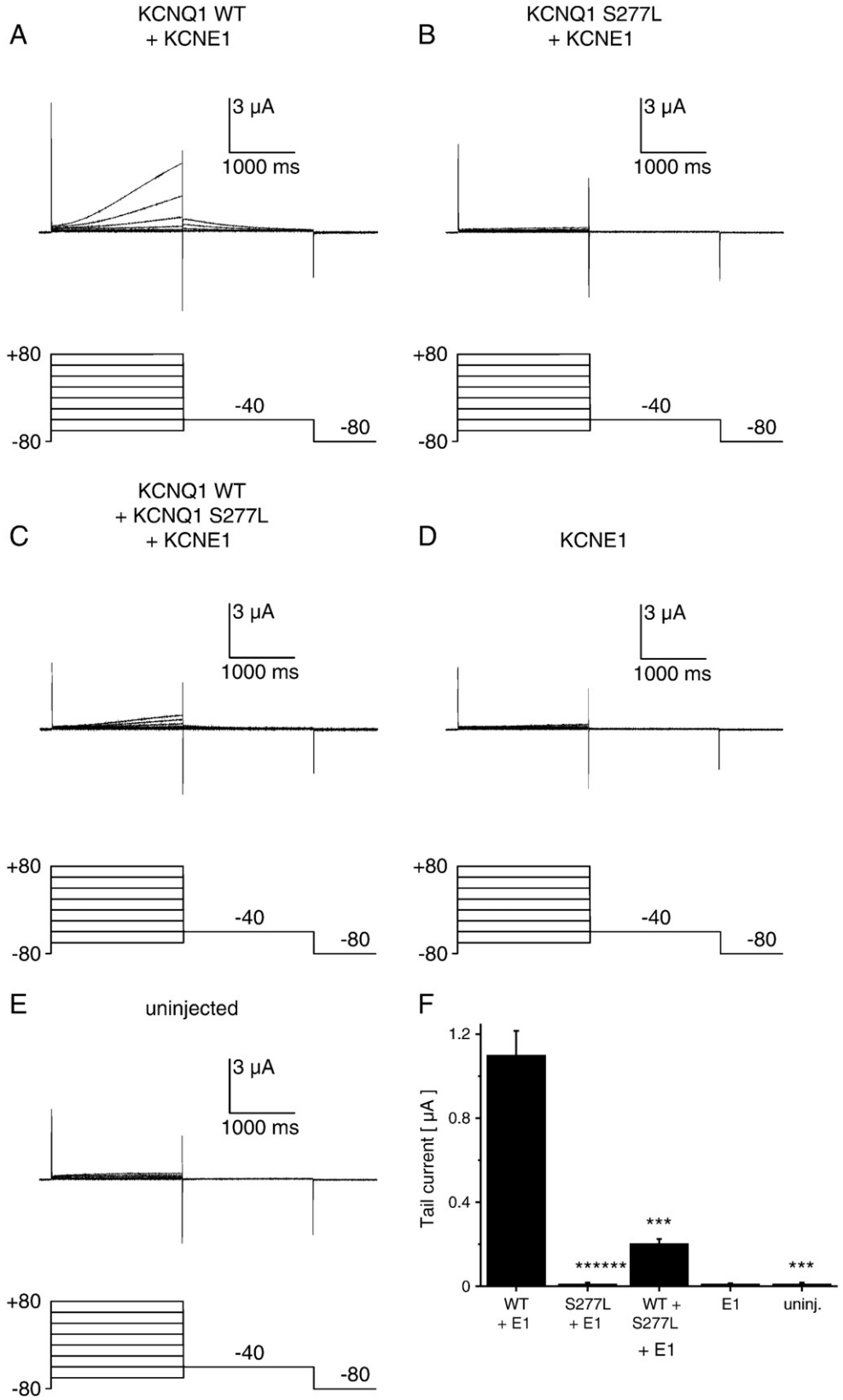


Fig. 3. Expression of I_{Ks} currents. I_{Ks} was generated by co-expression of WT KCNQ1 (A) or KCNQ1 S277L (B) in combination with KCNE1 cRNA. (C) I_{Ks} currents resulting from co-expression of KCNE1 with equal amounts of WT KCNQ1 and KCNQ1 S277L subunits. (D) currents induced by expression of KCNE1 β-subunits. (E) currents recorded from a non-injected control cell. (F) mean peak tail current amplitudes (n = 36 cells per column; $^{***}p<0.001$ compared to WT KCNQ1 + KCNE1). For the purpose of clear presentation, measurements obtained at 100 mV and 120 mV are omitted from panels A to E.

is supported by additional studies that did not reveal a consistent correlation between KCNQ1 genotype and clinical phenotype [10,15,17]. Limitations of this study arise from the fact that genetic sequencing was not performed in additional ion channel genes to screen for second mutations after the mutation in KCNQ1 was identified in our patients. Thus, some of the S277L mutation carriers

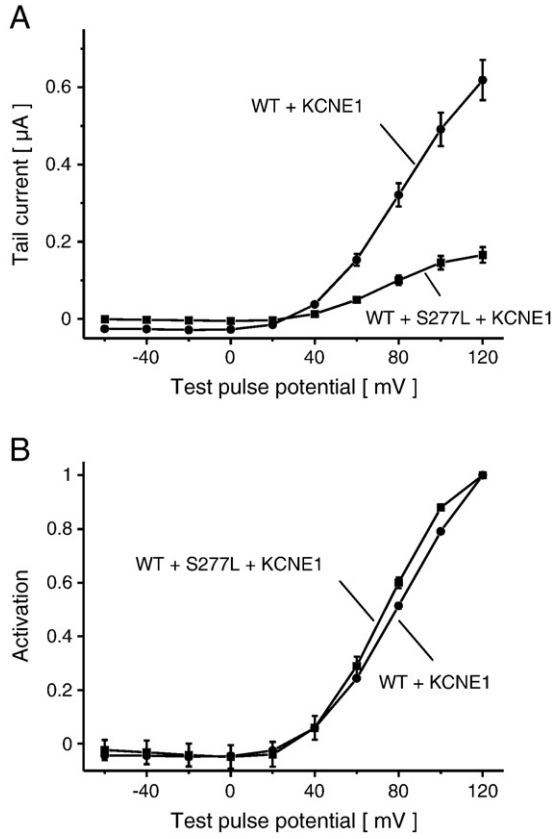


Fig. 4. I–V relationships for I_{Ks} peak tail currents shown in Fig. 3. (A) mean current amplitudes. (B) normalized current amplitudes. Mean values ± SEM are shown (n = 15).

may have carried a second mutation, potentially modifying the clinical phenotype.

Mutations of KCNQ1 S277 have been reported previously (ΔS277, S277L, S277W [19,20,26,27]). However, detailed clinical and/or biophysical data are limited. Liu et al. [26,27] reported four S277L mutation carriers, of which two died suddenly (QTc = 540 and 680 ms, respectively) and two remained asymptomatic (QTc = 470 ms in both subjects). In contrast to the index patient reported in our study, the affected patients died following exercise and excitement which more frequently triggers arrhythmias in LQTS1 than emotional stress. In summary, these clinical observations confirm high phenotypic variability associated with KCNQ1 S277 mutations.

In conclusion, the KCNQ1 S277L mutation causes long QT syndrome 1. The clinical severity of KCNQ1-associated long QT syndrome 1, however, does not solely depend on the disease-causing mutation itself. Rather, it appears to be determined by additional factors, such as compound mutations, low penetrance, and the individual repolarization reserve, comprising genetic, epigenetic, hormonal, and environmental factors [14,28,29,30]. In particular, the KCNE1 D85N polymorphism was recently revealed to reduce I_{Ks} amplitudes [31]. Thus, phenotypic variability may depend on the presence or absence of KCNE1 polymorphisms in KCNQ1 S277L gene carriers.

Acknowledgements

We thank Ramona Bloehs and Katharina Ziegler for excellent technical assistance. This study was supported in part by grants from the Deutsche Forschungsgemeinschaft (FRONTIERS program to D.T.), from the ADUMED Foundation (to D.T.), from the German Heart Foundation/German Foundation of Heart Research (to D.T.), and from the Max-Planck-Society (TANDEM project to P.A.S.).

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